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TITLE OF INVENTION (280 characters max)

STEM CELL TREATMENT OF ACUTE RENAL FAILURE, MULTI-ORGAN FAILURE, AND EARLY DYSFUNCTION OF KIDNEY TRANSPLANT

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APPLICATION FOR UNITED STATES LETTERS PATENT

PROVISIONAL APPLICATION

INVENTOR: Christof Westenfelder, M.D.

TITLE: STEM CELL TREATMENT OF ACUTE RENAL FAILURE, MULTI-ORGAN FAILURE, AND EARLY DYSFUNCTION OF KIDNEY TRANSPLANT

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STEM CELL TREATMENT OF ACUTE RENAL FAILURE, MULTI-ORGAN FAILURE, AND EARLY DYSFUNCTION OF KIDNEY TRANSPLANT

FIELD OF THE INVENTION

The present invention generally relates to the field of therapies with bone marrow-derived stem cells for organ failure and more specifically relates to methods for treating and preventing renal dysfunction and multi-organ dysfunction including, but not limited to, acute renal failure of native kidneys, acute renal failure of native kidneys in the setting of multi-organ failure, multi-organ failure, acute renal failure in transplanted kidneys and to promote wound repair.

BACKGROUND OF THE INVENTION

Multi-organ failure (MOF) remains a major unresolved medical problem. MOF develops in the most severely ill patients who have sepsis, particularly when the latter develops after major surgery or trauma. MOF is characterized by shock, acute renal failure (ARF), leaky cell membranes, dysfunction of lungs, liver, heart, blood vessels and other organs. Mortality due to MOF approaches 100% despite the utilization of the most aggressive forms of therapy, including intubation and ventilatory support, administration of vasopressors and antibiotics, steroids, hemodialysis and parenteral nutrition. In addition, in many of these patients the healing of surgical wounds or those due to trauma and when infected, is seriously impaired, further contributing to recurrent infections, morbidity and death.

Patients with isolated ARF from any cause, i.e., ARF that occurs without MOF, continue to have a mortality in excess of 50%. This dismal prognosis has not improved despite intensive care support, hemodialysis, and the recent use of atrial natriuretic peptide, Insulin-like Growth Factor-I (IGF-I),

more biocompatible dialysis membranes, continuous hemodialysis, and other interventions.

ARF or early graft dysfunction (EGD) is a common complication in patients who receive a kidney from a cadaveric donor. EGD results from the ischemic injury of the donor kidney that collectively results from harvesting, transportation and actual implantation of the allograft ("cold" and "warm" ischemia). EGD or transplant-associated ARF has its own immediate morbidity, mortality and can occasionally lead, i.e., within a few days of transplantation, to the permanent loss of the grafted kidney. Later adverse consequences resulting from significant EGD, despite return of adequate renal function, include much higher rates of graft rejection and complications caused by the repeated administration of anti-rejection medications, and often premature loss of the transplant, necessitating resumption of dialysis support.

Taken together, therapies that are currently utilized in the prevention of ARF, the treatment of established ARF of native kidneys per se or as part of MOF, and ARF of the transplanted kidney, have not succeeded to significantly improve morbidity and mortality in this large group of patients. Consequently, there exists an urgent need for the improved treatment of MOF and ARF.

Very promising pre-clinical studies in animals and a few early phase clinical trials administer bone marrow-derived hematopoietic stem cells for the repair or protection of one specific organ such as the heart, small blood vessels, brain, spinal cord, liver, etc. These treatments use only a single population of bone-marrow stem cells, either Hematopoietic (HSC) or Mesenchymal Stem Cells (MSC), and obtained results are very encouraging in experimental stroke, spinal cord injury, myocardial infarction and other conditions. MSC have been infused to patients a few weeks after they first received a bone marrow transplant in the treatment of cancers, osteogenesis imperfecta, and Hurler's syndrome, situations in which MSC infusions accelerated reconstitution of adequate hematopoiesis, and in which they were furthermore effective in the treatment of osteogenesis imperfecta and Hurler's syndrome, respectively. Importantly, however, simultaneous administration of

HSC and MSC, known to physiologically cooperate in the maintenance of hematopoiesis in the bone marrow, has, until now (our studies, see below) not been utilized for the treatment of any of the above listed disorders.

In ARF (native kidneys, transplanted kidney), microvascular endothelial cells and proximal as well as distal tubular cells are destroyed and become dysfunctional when injured, insults that together mediate the acute loss of kidney function. Successful recovery from ARF depends directly on the repair of injured renal vessels and tubular segments. Since both HSC and MSC possess a remarkable level of plasticity, i.e., are capable to differentiate into several non-hematopoietic cell types (neurons, heart, muscle, liver, vascular and other cells) including renal tubular and vascular endothelial cells, pre-clinical studies were begun based on the concept that the co-administration of HSC and MSC may be more effective as it reproduces their mutually supportive capacity in the bone marrow. In pre-clinical studies, rats with ARF were treated with a defined mix of autologous HSC and MSC. As discussed below, the data demonstrate that this novel intervention is highly effective in the prevention of ARF, the reestablishment of normal renal function (see below) and elimination of animal mortality. Results obtained with the co-administration of HSC and MSC in ARF are generally superior to those obtained with either HSC or MSC alone.

DETAILED DESCRIPTION OF THE INVENTION

The present invention will utilize stem cells for the repair of critically damaged tissues. In one aspect of the present invention, autologous hematopoietic stem cells (HSC) and mesenchymal stem cells (MSC) are co-administered to a patient in need thereof in defined ratios. The co-administration of HSC and MSC may be used in the treatment of multi-organ failure, acute renal failure of native kidneys, ARF of native kidneys in multi-organ failure, or ARF in transplanted kidneys. Defined ratios of HSC and MSC may be used to treat the dysfunction of other organs, such as lungs, liver, heart, or poorly healing wounds. Autologous MSC and HSC may also be

administered in defined ratios to be utilized as a preventative measure, for example in patients at risk for developing multi-organ failure, all types of ARF, and to promote wound healing.

HSC for administration are obtained, by aspiration under local anesthesia, from the bone marrow of patients to be treated for ARF, i.e., are autologous, or, under defined circumstances, may be collected from a compatible donor. The HSC are isolated from a donor or the patient themselves by techniques commonly known in the art. In a preferred embodiment of the present invention, autologous HSC are isolated and administered. The pluripotent HSC population is enriched after harvest from the bone marrow using fluorescence activated cell sorting (FACS), selecting for "c-kit" positive, "sca-1" positive and "lin negative" cells. "c-kit' and "sca-1" cells are known to one of skill in the art as being receptors known to be on the surface of stem cells. A "lin negative" cell is known to one of skill in the art as being a cell that does not express antigens characteristic of specific cell lineages and thus is more primordial, pluripotent and able of self-renewal. The HSC may be CD 34 positive or negative. Any method known to one of skill in the art may be used to enrich the population of pluripotent stem cells from the whole population of bone marrow cells, and, if necessary, cryopreserve them until needed for therapy. Alternatively and time permitting, autologous HSC may be obtained from the peripheral blood by using routine HSC mobilization protocols and repeated leukapheresis, HSC enrichment by FACS, and cryopreservation of HSC until use. Mobilization of HSC into the peripheral circulation is accomplished by the daily administration of G-CSF alone or in conjunction with cytoxan or SCF. The resultant increase in peripheral leukocytes is paralleled by an increase in circulating HSC numbers which are collected by repeated leukapheresis, as is routinely done in patients who receive an autologous bone marrow transplant after having undergone myeloablative chemotherapy or radiation for the treatment of various malignancies. This "slower" approach of collecting HSC may be best suited for those patients who are scheduled to undergo an elective high risk surgery,

i.e., patients in whom there is sufficient time to collect HSC in this fashion, while their MSC are conventionally obtained from their bone marrow aspirate (see below).

MSC for administration are derived from bone marrow cells that are placed into sterile culture *in vitro*. Except for MSC, practically all other cells contained in a bone marrow aspirate will not adhere to the bottom of a culture dish (Friedenstein, *Exp Hematol* 4:267-74, 1976). After discarding the non-attached cells, MSC will grow and expand in culture, yielding a well defined population of pluripotent stem cells. After expansion in vitro, collected MSC may be further depleted of CD 45 positive cells, by FACS, in order to remove residual macrophages or other hematopoietic cell lineages prior their administration to the patient. MSC may be derived from the patient or, under defined circumstances, from a compatible donor. Donor stem cells may be used from a donor having similar compatibility as defined for the organ to be transplanted, known to one skilled in the art. Since MSC can be expanded in vitro, the treatment regime with MSC can be easily repeated in order to further augment the cellular repair processes in the injured kidney. Any method known to one of skill in the art may be used to enrich the population of pluripotent MSC from the whole population of bone marrow cells, and, if necessary, cryopreserve them until needed for therapy.

Use of autologous MSC and HSC eliminates concerns regarding immune tolerance. Additionally, repetitive administrations of autologous MSC and HSC are possible.

Allegoric MSC and HSC, derived from a compatible donor may also be used for co-administration. Reasons for administering allogenic stem cells include: (a) the bone marrow in a patient who is found to need stem cell therapy for ARF etc. may be a poor source of adequate numbers of stem cells because the patient may have received bone marrow toxic drugs or radiation or may have bone marrow cancer; (b) a patient may refuse or may not be able to consent to the harvesting of his/her own bone marrow cells; (c) the bone marrow-derived stem cells from a compatible living-related or unrelated donor

of a solid organ may be of superior quality and quantity compared to the recipient's own stem cells; (d) bone marrow-derived stem cells alone from a compatible living donor of bone marrow only, not a kidney, for the treatment of ARF etc. may be of superior quality and quantity compared to that of the recipient's own stem cells.

Co-administration of MSC and HSC includes simultaneous administration of MSC and HSC, administration of MSC followed by administration of HSC and administration of HSC followed by administration of MSC. The time interval between the sequential or repeated administration of HSC and MSC, respectively, is generally, if utilized, is generally 1-2 days or a few weeks, depending on the responses that are obtained or expected.

In certain embodiments, a therapeutically effective dose of stem cells is delivered to the patient. An effective dose for treatment will be determined by the body weight of the patient receiving treatment, and may be further modified based on the severity of ARF, the phase of ARF in which therapy is initiated, and the simultaneous presence or absence of MOF. A therapeutic dose may be one or more administrations of the therapy. Additionally, a subsequent therapeutic dose may include a therapeutic dose of HSC and MSC, HSC alone, or MSC alone.

The ratio of HSC to MSC for administration for treatment may be greater than about 3:1, greater than 4:1, greater than 5:1, greater than about 6:1, greater than about 7:1, greater than about 8:1, less than about 8:1, less than about 7:1, less than about 6:1, less than about 5:1, less than about 4:1, less than about 3:1, about 1:1, more preferably in the range of about 3:1 to about 8:1, most preferably about 5:1. Different ratios from those above may prove more effective at certain stages of ARF, e.g. early vs. late after onset. Different ratios may be used for treatment of different or more complex disorders, including MOF. Ratios of may be about 0.1:1 to about 50:1, depending on the disease being treated.

Stem cells are administered to the patient by injection or instillation intravenously (large central vein such vena cava) or intra-arterially (via

femoral artery into supra-renal aorta). Any delivery method for stem cells, commonly known in the art, may be used for delivery of the co-administered MSC and HSC.

In our Pre-Clinical Studies, several methods are used to track administered HSC/MSC in the kidney and other organs such as the liver, heart or brain. Cell tracking systems may be used in which HSC and MSC are labeled with vital dyes prior to administration. These vital dyes, i.e., dyes that have no harmful effect on living cells, allow the precise location of administered HSC/MSC in the kidney or any organ, using techniques commonly known in the art. Another system that may be utilized to track HSC and MSC in experimental models uses HSC and MSC from syngeneic animals that are transgenic for human Placental Alkaline Phosphatase (hPAP) or enhanced Green Fluorescent Protein (eGFP). The administered HSC and MSC from such transgenic donor animals can be readily identified in wild-type recipients of the same animal strain, using techniques commonly known in the art for identifying cells expressing hPAP or eGFP. In addition, in experimental models as well as in human subjects, when HSC or MSC are derived from a male animal or male human donor, and when these are administered to a female animal or female human recipient, the presence of the male "Y" chromosome in the donor cells that are engrafted in the recipient's target organs or found in the circulation can be identified by Fluorescent In Situ Hybridization (FISH assay), RT-PCR and immunocytochemistry.

Besides the tracking of administered HSC/MSC, their post-infusion differentiation into kidney-specific or other defined cells of injured organs must be confirmed in pre-clinical studies. For example, in the kidney, demonstration that the infused HSC/MSC have differentiated into the renal cell type that needs to be reconstituted is accomplished by assaying administered HSC/MSC for the de novo expression of cell markers that are specific for distinct kidney cell types, e.g., proximal tubular and microvascular endothelial cells. This double labeling technique, i.e., cell tracking and proof of target organ-specific differentiation, provides conclusive evidence as to the

origin (HSC/MSC) and kidney-specific phenotype (proximal tubular and vascular endothelial cells) that these cells have differentiated into, respectively.

In the bone marrow, as well as in long-term in vitro cultures, MSC support growth of HSC and HSC interact with MSC. Both cell types are capable of differentiation, to a variable degree, into various non-hematopoietic cell types, including renal, vascular, neuronal, myocardial, hepatic and others. Co-administration of these mutually supportive MSC and HSC conceptually reproduces the situation in the bone marrow, potentially facilitating more efficient engraftment and differentiation of these cells into those that are destroyed in ARF, i.e., carrying out the repair of microvascular and tubular injuries. Furthermore, ARF causes low level mobilization of HSC from the bone marrow into the peripheral circulation, thus suggesting that therapeutic augmentation of the delivery of both stem cell types to the injured kidney in ARF may be superior to the treatment with only HSC or MSC, respectively. Microenvironmental changes that are created by vascular and tubular cell injury in ARF generate homing and differentiation signals for stem cells, signals that guide and regulate the repair processes, thought to be primarily carried out by surviving renal cells. Co-administration of MSC and HSC may, through a transient mechanism, further protect organ function and augment organ repair because these cells can locally release growth factors and cytokines such as Hepatocyte Growth Factor (HGF) and others. Their intrarenal release in para-, auto-, and endocrine fashion, may be particularly beneficial in the early phase of ARF treatment with stem cells, since some of the growth factors enhance cell survival and stimulate proliferation of renal cells in ARF, both responses that can protect and improve renal function. Subsequent progressive differentiation of MSC and HSC into kidney-specific cell types engrafted at sites of renal injury, will directly contribute to or undertake the necessary cellular repairs.

In addition to providing kidney precursor cells in the treatment of ARF, co-administered MSC and HSC may be utilized for therapeutic gene delivery.

MSC, HSC, or both MSC and HSC may be transfected with genes prior to their administration to a patient. The transfected genes may include genes whose products are known to support cellular survival, stimulate cell migration and proliferation, to exert anti-inflammatory actions and to improve intrarenal hemodynamics. The activity of genes delivered in this fashion may be placed under the control of drug-sensitive promoters that allow both controlled activation and inactivation of these genes. The term "genetically transformed" as used herein refers to stem cells that have been genetically modified with exogenous DNA or RNA. The term "transformed" refers to cells that have acquired malignant characteristics. The term "non-transformed" refers to stem cells that have not been genetically modified with exogenous DNA or RNA.

Defined patient populations are expected to benefit from the administration of HSC/MSC. For example, patients with treatment-resistant (hemodialysis, parenteral nutrition, antibiotics, ICU care) forms of ARF alone or in the setting of MOF or multi-organ dysfunction, have only a small survival chance and will therefore be the prime candidates for this cell-based treatment. Patients at highest risk for or who are about to develop the most severe form of treatment-resistant ARF would be prepared for HSC/MSC therapy by obtaining their bone marrow aspirate and preparing HSC/MSC cells as above. Time permitting, HSC may be obtained with a stem cell mobilization and leukapheresis protocol as detailed above. The prepared cells may be frozen and only administered when warranted by a very poor outcome such as life threatening deterioration in the function of kidneys and/or other vitally important organs. Additionally, trauma or surgical patients, scheduled to undergo high risk surgery such as the repair of an aortic aneurysm, may also benefit from prophylactic HSC/MSC collection and preparation prior to major surgery. In the case of poor outcome, including infected and non-healing wounds, development of MOF post surgery, the patient's own HSC/MSC that are cryopreserved may be administered.

Patients with severe ARF affecting a transplanted kidney may either be treated with HSC/MSC from the donor of the transplanted kidney (heterologous) or with cells from the recipient (autologous).

In another aspect of the present invention, MSC and HSC may be co-administered in defined ratios for the treatment of MOF and the ARF that always develops in patients with MOF. MSC and HSC therapy in the setting of MOF would likely be able to contribute to and augment, in a systemic sense, the repair process of all significantly injured organs, i.e., kidneys, lungs, heart, liver, etc. It has been shown that the administration of bone marrow-derived stem cells to animals with damage of very different organs, e.g., experimental stroke or other models of neurotoxicity, spinal cord injury, myocardial infarction, liver injury, and our own data in ARF, results in protection and repair of individually targeted organs. Co-administration of MSC and HSC, as described in this invention, may thus represent an intervention that can effectively boost a patient's capacity to carry out repair of any damaged organ, and thereby ameliorate or overcome the many deleterious consequences of MOF.

In another aspect of the present invention, MSC and HSC may be co-administered in defined ratios for the treatment of ARF in the transplanted kidney. MSC have been shown to act in an immunomodulatory manner, i.e., they are able to enhance a recipient's tolerance for an allograft. Administration of bone marrow-derived stem cells from the kidney donor results in generalized microchimerism in the kidney recipient, also known to lead to enhanced graft tolerance. Co-transplantation of MSC and HSC may have immediate renoprotective effects, as in ARF of native kidneys (see above), thereby ameliorating or preventing EGD, as well as diminishing the late consequences of severe EGD (increased graft rejection rates) by induction of enhanced graft tolerance through several immune-modulatory mechanisms (see above). In addition, administration of autologous HSC/MSC, obtained from the kidney recipient, may permit significant replacement of donor renovascular endothelial cells with those of the recipient. This appears

feasible since it is known that HSC/MSC are able to differentiate into vascular endothelial cells and since these are able to engraft into the damaged vasculature of the ARF kidney. Replacement of donor renovascular endothelial cells that are lost in EGD with endothelial cells derived from autologous MSC and HSC, may thus reduce the immunogenicity of the donor kidney, since vascular endothelial cells represent the most immediate barrier between the recipient's blood elements, including cells and antibodies that mediate vascular/cellular rejection, and the parenchymal cells of the implanted kidney. Accordingly, replacement of a significant percentage of the donor kidney's vascular endothelial cells with endothelial cells derived from the recipient's MSC and HSC will reduce the number of "foreign" vascular endothelial cells present in the transplant, creating potentially a form of "renovascular microchimerism".

Additionally, "renovascular microchimerism" (see above) may be more rapidly accomplished by administering, upon reperfusion of the implanted kidney, autologous vascular endothelial cells that are obtained by in vitro pre-differentiation of the recipient's HSC and MSC. These pre-differentiated cells can be prepared in advance of the kidney transplant, and are cryopreserved until the time of the transplant.

In another aspect of the present invention, the cellular repair processes in ARF or MOF may be significantly accelerated when the cells administered to the patient are pre-differentiated in vitro from HSC and MSC, as described above. Administration of vascular endothelial cells exerts renoprotective effects in ischemic ARF, as describe below. HSC and MSC can differentiate both into renal tubular and vascular cells. Therefore, the cellular repair processes may be further accelerated when administered cells are pre-differentiated in vitro (from HSC and MSC) into endothelial and/or renal tubular or cells of other organs. With this technique, an injury of kidney or other organs may be organ- and cell-specifically treated. In MOF, micro-vascular and parenchymal injuries of other organs parallel those seen in ARF. It appears that organ injury is associated with low level mobilization of HSC.

In both multi-organ failure and ARF the low level mobilization of HSC may be inadequate to effectively aid in the repair of severely injured organs. Therefore, replacement of vascular endothelial cells, derived from HSC and MSC, combined with organ-specific pre-differentiated renal or other parenchymal cells (the chosen cell type is determined by the organ with the most life threatening dysfunction), may be highly effective in improving organ function and patient/animal survival in MOF.

In another aspect of the present invention, autologous or heterologous hemangioblasts, a subgroup of HSC, are selected by FACS from autologous or heterologous HSC and may be used for the treatment of MOF, acute renal failure of native kidneys, ARF of native kidneys in multi-organ failure, and ARF in transplanted kidneys. Ischemic injury of various organs results in the spontaneous appearance of hemangioblasts through their mobilization from the bone marrow into the peripheral circulation. These cells express in humans a characteristic cell surface antigen (CD 133 or AC 133), often in conjunction with CD 34, a common stem cell marker, allowing their enrichment with FACS sorting. In mice and rats, vascular endothelial cell precursors or hemangioblasts express the KDR receptor for Vascular Endothelial Growth Factor (VEGF), also facilitating enrichment by FACS sorting. Upon differentiation into endothelial or hematopoietic cells, CD 133 and KDR expression disappears. Hemangioblasts are capable of supporting both vasculogenesis/angiogenesis and hematopoiesis. These characteristics may be particularly desirable when there is severe vascular injury and poor wound healing.

In another aspect of the present invention, the above delineated technologies may be established in tertiary care centers world wide. In analogy to company-owned in-hospital and free-standing hemodialysis units, multidisciplinary "Nephroplasty" or "Cell Therapy Teams" could be owned and operated by international Health Care Equipment and Service companies that would also produce and sell their or other companies' kits and materials used for the harvesting, purification, culturing, differentiation, cryopreservation and

administration of SCs or SCs that are pre-differentiated in vitro to patients at high risk for ARF or multi-organ failure (Prevention), and to patients with established ARF or multi-organ failure (Treatment). Physicians (Nephrologists, Intensivists, etc.) who care for this group of patients would order respective cell-based services, and these specialized teams would provide the requested treatment.

In a preferred embodiment of the present invention, the stem cells utilized for these treatments will be "harvested" and prepared on site, i.e., in the hospital by a specialized team from the following donors: 1) a patient will donate his/her own bone marrow for treatment of his/her own ARF or organ failure, i.e., autologous stem cells or 2) a compatible but allogeneic donor, i.e., not tissue-type identical donor, when ARF develops in the transplanted kidney or organ failure develops in another transplanted organ (heart, liver, lungs). In this setting, harvesting of solid organs from a cadaveric donor (kidneys, liver, heart etc.) would be complemented by the simultaneous harvesting of the cadaveric donor's bone marrow-derived stem cells, by that very same specialized team (as above). Since the solid organs to be transplanted are always screened, by tissue typing, for compatibility with prospective recipients, the simultaneously harvested stem cells would thus be automatically identified as being compatible with the recipient of any of the solid organs. Thus, keeping these stem cells available by cryopreservation, makes them readily available for developing treatment needs following transplantation of the solid organs into multiple recipients (kidneys, heart, liver, etc.).

EXAMPLES

Example 1

Determine the Ratio of MSC and HSC for Co-Administration Therapy

Using as a guideline the approximate ratio of HSC and MSC numbers in the normal bone marrow, protocols in which the ratios or doses of co-

administered HSC/MSC given to rats with ARF were varied. In preliminary experiments, adult Sprague-Dawley or Fisher 344 rats (male or female) were studied. Ischemia/reperfusion-type of ARF ("ischemic ARF") is induced in anesthetized rats by timed clamping of both renal pedicles, thereby interrupting the blood supply to the kidneys causing an "ischemic" insult that results in acute loss of kidney function, i.e., ARF. This model of ARF very closely resembles the most common and most serious form of ARF in patients with shock, sepsis, trauma, after vascular surgery, etc.

The relative renoprotective potency of various SC treatment protocols was tested by infusing intravenously (jugular, femoral or tail vein) or intra-arterially (into aorta via carotid or femoral artery) HSC alone, MSC alone or HSC in combination with MSC at a HSC/MSC ratio of 1:1, 3:1, 5:1 or 8:1 to rats immediately after induction of ARF as well as infusion of HSC alone, MSC alone or HSC/MSC in ratios of 1:1, 3:1, 5:1 or 8:1 24 hrs after induction of ARF. The total number of cells administered in all studies was about 10^5 to 10^6 cells/animal.

Renal function in the experimental model was monitored, as in patients, by determination of blood creatinine and BUN levels, measurement of creatinine clearance and urine output. Overall outcome was assessed by determination of weight loss, hemodynamics, and survival. After sacrifice of control and HSC/MSC-treated animals with ARF, kidneys were examined for the degree of histological injury (cell apoptosis, necrosis, vascular congestion and injury, inflammatory cell infiltrates) and repair (mitogenesis, redifferentiation of cells, decongestion, etc.), intrarenal localization of the administered HSC/MSC (as discussed above, the administered HSC/MSC are tagged for tracking purposes), and their integration and differentiation into renal cells.

The following observations from the preliminary experiments using the rat model of ARF were made. Outcome is greatly improved when HSC/MSC are administered in combination at an average HSC/MSC ratio of 5:1. Animal mortality was abolished with the combination treatment. In comparison, HSC

or MSC given individually provide a modest renoprotective effect. Stem cell engraftment, differentiation and integration into the ARF kidney occurred with much greater efficiency when HSC and MSC were co-administered, explaining the excellent organ repair and functional recovery that is obtained. Additionally, there were no adverse effects that resulted from the administration of HSC, MSC or both together, since these were autologous (from the animal or potentially the patient who is treated for ARF) or "syngeneic" cells (from the animal's litter mate or a HSC/MSC donor who is immune-compatible with the patient who is treated for ARF).

Additional studies will be conducted to optimize co-administration protocols, including using different ratios of stem cells, and to further identify and augment intrarenal homing and differentiation signals.

The results from the experiments in the rat model will be applicable to the treatment of patients. In clinical practice, patients who qualify for this form of treatment, i.e., those with the severest form of ARF, one that carries a mortality of up to 100%, particularly when ARF develops in the setting of multi-organ failure, will serve as their own, autologous HSC/MSC donors. Bone marrow is aspirated under local anesthesia and under sterile conditions. HSC are isolated and enriched from the bone marrow aspirate using FACS and are subsequently cryopreserved until use. Highly pure MSC are generated in sterile culture of bone marrow aspirates. Appropriate numbers of HSC and MSC are combined at a defined ratio, e.g., 5:1, suspended in sterile saline or McCoy' solution, and administered into a large central vein. The latter access is always established in this group of patients. Unless contraindicated, a suprarenal aortic route of administration may prove superior, and can be routinely accomplished by cannulating a femoral artery and advancing the tip of the infusion catheter to an intra-aortic location well above the renal arteries. This route of administration allows the most direct and SC dose-sparing delivery of HSC/MSC into both renal arteries and thus into both kidneys. Studies in which the therapeutic results that are obtained with the intravenous infusion route (superior vena cava) are compared with

those obtained using the intra-aortic route will establish which approach is superior. It is also important to note that, if needed, treatments with autologous HSC/MSC can be repeated. And, HSC and MSC from the donor of a kidney whose tissue type is close enough to that of the recipient and thus permits a successful heterologous transplant, thereby requiring no or only modest immunosuppresive therapy, may also be administered at the time of or following the kidney transplant, for the prevention and treatment of EGD, respectively.

Example 2

Determine the Ratio of MSC and HSC for Co-Administration for Wound Healing

The administration of MSC and HSC mixes to rats with ARF resulted in improved outcome (see above). Also, the abdominal, well-healed incision initially created for the induction of ARF (clamping of both renal arteries), contained large numbers (~ 40%) of MSC and HSC-derived vascular and other cells, indicating that MSCs and HSCs can powerfully support the process of wound healing that includes angiogenesis. Further studies in animals with experimental abdominal wound infections alone or in the setting of LPS-induced shock with MOF, will examine whether SC therapy, as defined above, improves wound healing and related outcomes (see Example 3).

Example 3

Determine Stem Cell Therapy for Multi-Organ Failure

Stem cell therapies will be investigated that may effectively boost the body's ability to cope with the many deleterious consequences of multi-organ failure and to carry out repair and functional recovery of multiple organs rather than that of a single one. The multi-organ failure model that will be used is the endotoxin model in mice, in which endotoxin from gram negative bacteria

(LPS) is injected, resulting in all the manifestations of clinical multi-organ failure, including ARF. Besides improvement in organ function, successful MSC and HSC therapy is expected to reduce the 100% mortality seen in experimental multi-organ failure, and to significantly enhance wound repair, when applicable (see Example 2 above).

Example 4

Determine MSC and HSC Therapy for generalized Microchimerism

Interventions to establish generalized microchimerism in order to induce increased immune tolerance of the transplanted kidney, i.e., reduced rejection rates, will be examined using suitable rat and mouse kidney transplant models, and employing autologous and heterologous donor and recipient combinations. The HSC and MSC will be co-administered in various ratios. HSC/MSC pre-differentiated in vitro or hemangioblasts will also be administered in separate experiments. The degree of microchimerism is determined by identification of tagged donor cells in the circulation, bone marrow and kidney, when applicable. The degree of graft acceptance or tolerance is tested in animals with heterologous transplants by tapering or discontinuing antirejection medications. Animals with microchimerism are expected to exhibit lower rejection rates than those without.

Example 5

Determine MSC and HSC Therapy for "Renovascular Microchimerism"

Interventions to establish "renovascular microchimerism" in order to induce increased immune tolerance of the transplanted kidney, i.e., reduced rejection rates, will be examined using suitable rat and mouse kidney transplant models, and employing autologous and heterologous donor and recipient combinations. The HSC and MSC will be co-administered in various

ratios. HSC/MSC pre-differentiated in vitro or hemangioblasts will also be administered. The degree of microchimerism is determined by identification of tagged donor cells in the circulation, bone marrow and kidney vasculature. The postulated degree of enhanced graft tolerance as a function of "renovascular microchimerism" is assessed as in Example 4 above.

Example 6

Determine therapeutic effectiveness of Hemangioblasts in ARF and MOF

Following the experimental design protocols identified above, isolated hemangioblasts from the bone marrow will be enriched in vitro and administered to prevent or treat ARF (native kidneys, transplanted kidney) and multi-organ failure. The very high potential of these cells to differentiate into vascular endothelial cells may prove to be particularly advantageous when renovascular or generalized vascular injury predominates in a particular phase of ARF of multi-organ failure. Results obtained with hemangioblasts will be compared to those obtained with protocols detailed in the preceding Examples.

CLAIMS

1. A method of treating organ dysfunction, treatment of acute renal failure, multi-organ failure, and early dysfunction of kidney transplant, said method comprising delivering a therapeutic amount of adult hematopoietic stem cells and adult mesenchymal stem cells to a patient in need thereof.

2. The method of claim 1 wherein said adult stem cells comprise autologous cells.

3. The method of claim 1 wherein said stem cells are delivered to said patient in a hematopoietic to mesenchymal stem cell ratio that is optimized for the treatment of ARF or other organ dysfunction.

4. The method of claim 3 wherein said stem cells are delivered to said patient in a ratio of about 0.1:1 to about 50:1 hematopoietic stem cells to mesenchymal stem cells.

5. A method of treating acute renal failure, multi-organ failure, and early dysfunction of kidney transplant, said method comprising delivering a therapeutic amount of pre-differentiated stem cells to a patient in need thereof;

wherein said cells are pre-differentiated in vitro into kidney- or other organ-specific cells.

6. A method of treating acute renal failure, multi-organ failure, and early dysfunction of kidney transplant, said method comprising delivering a therapeutic amount of hemangioblasts to a patient in need thereof.

7. The method of claim 6 wherein said hemangioblasts comprise autologous cells.

8. The method of claim 7 wherein said hemangioblasts comprise allogeneic cells.

9. A system for the treatment of patients comprising a multidisciplinary cell therapy team to administer stem cell therapy to patients in need thereof; wherein said stem cell therapy team harvests, processes and delivers a therapeutic amount of adult hematopoietic stem cells and adult mesenchymal stem cells or pre-differentiated stem cells or hemangioblasts.

10. The system of claim 9 wherein a health care company supports or owns said cell therapy team.

11. The system of claim 10 wherein said health care company provides materials and kits needed for the harvesting, purification, culturing, differentiation, cryopreservation and administration of stem cells.

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